

C2
1. (three times amended) An *in vitro* system that recapitulates regulated RNA deadenylation and degradation of an exogenously added preselected target 3' polyadenylated messenger RNA sequence comprising

i) a cytoplasmic extract supernatant from a 100,000 x g, 1 hour centrifugation isolated from eukaryotic cells or tissues, said extract depleted of activity of proteins that bind polyadenylate;

ii) a source of ATP; and

iii) an exogenous target 3' polyadenylated messenger RNA sequence.

Subst E5
C3
~~4. (amended) The system of claim 3 wherein said cytoplasmic extract is obtained from a cell line selected from the group consisting of HeLa cells and a T cell line.~~

6
~~5. (amended) The system of claim 1 wherein said cytoplasmic extract is prepared from cells comprising foreign nucleic acid.~~

7
~~6. (amended) The system of claim 1 wherein said cytoplasmic extract is prepared from cells which are infected, stably transfected, or transiently transfected.~~

C4
~~9. (amended) The system of claim 1 wherein said cytoplasmic extract is selected from the group consisting of:~~

(a) a cytoplasmic extract which contains polyadenylate competitor RNA;

(b) a cytoplasmic extract which contains a material that sequesters proteins that bind polyadenylate;

(c) a cytoplasmic extract which contains a proteinase that inactivates a protein that bind to polyadenylate; and

(d) a cytoplasmic extract which contains an agent that prevents the interaction between polyadenylate and an endogenous macromolecule that binds to polyadenylate.

10. (amended) The system of claim 9 wherein the material that sequesters proteins that bind polyadenylate is selected from the group consisting of 1. antibodies to proteins that bind polyadenylate, 2. polyadenylate, and 3. a combination of antibodies to proteins that bind polyadenylate, and polyadenylate.

14. (amended) The system of claim 1 wherein said target 3' polyadenylated messenger RNA sequence is selected from the group consisting of an unlabeled 3' polyadenylated messenger target RNA sequence, a labeled 3' polyadenylated messenger target RNA sequence, and a [the] combination thereof.

15. (amended) The system of claim 14 wherein said labeled target 3' polyadenylated messenger RNA sequence is labeled with a moiety is selected from the group consisting of a fluorescent moiety, a visible moiety, a radioactive moiety, a ligand, and a combination of fluorescent and quenching moieties.

17. (amended) The system of claim 1 wherein said source of ATP is exogenous.

21. (amended) A method for identifying an agent capable of modulating the stability of a target 3' polyadenylated messenger RNA sequence comprising
(A) providing the system of claim 1;
(B) introducing said agent into said system;
(C) determining the extent of deadenylation and degradation of said target 3' polyadenylated messenger RNA sequence; and
(D) identifying an agent able to modulate the extent of said turnover as capable of modulating the stability of said target 3' polyadenylated messenger RNA sequence.

23. (amended) The method of claim 1 wherein said source of ATP is exogenous.

17 ¹⁵
25. (amended) The method of claim 21 wherein said target 3' polyadenylated messenger RNA sequence is selected from the group consisting of an unlabeled target 3' polyadenylated messenger RNA sequence, a labeled target 3' polyadenylated messenger RNA sequence, and a combination thereof.

Subst
E10
~~26. (amended) The method of claim 25 wherein said labeled target 3' polyadenylated messenger RNA sequence is labeled with a moiety is selected from the group consisting of a fluorescent moiety, a visible moiety, a radioactive moiety, a ligand, and a combination of fluorescent and quenching moieties.~~

27. (amended) The method of claim 21 wherein said monitoring the extent of turnover of said target 3' polyadenylated messenger RNA sequence comprises determining the extent of degradation of said labeled target 3' polyadenylated messenger RNA.

21 ¹⁵
~~28. (amended) The method of claim 21 wherein said modulating the stability of a target 3' polyadenylated messenger RNA sequence increases the stability of said target RNA sequence.~~

22 ¹⁵
~~29. (amended) The method of claim 21 wherein said modulating the stability of a target 3' polyadenylated messenger RNA sequence decreases the stability of said RNA sequence.~~

24 ²³
30. The method of claim 30 wherein said AU rich element binding protein is selected from the group consisting of a member of the ELAV protein family; AUF1; tristetraprolin; AUH; TIA; TIAR; glyceraldehyde-3-phosphate; hnRNP C; hnRNP A1; AU-A; and AU-B.

33. (amended) A method for identifying an agent capable of modulating the stability of a target 3' polyadenylated messenger RNA sequence in the presence of an exogenously added RNA stability modifier comprising

- 34
Subst
E11
(a) providing the system of claim 1;
(b) introducing said RNA stability modifier into said system;

- C11
- (c) introducing said agent into said system;
 - (d) ~~determining the extent of turnover of said target 3' polyadenylated messenger RNA sequence; and~~
 - (e) identifying an agent able to modulate the extent of said turnover as capable of modulating the stability of said target 3' polyadenylated messenger RNA sequence in the presence of said exogenously added RNA stability modifier.
-

C12
Sub
E12
35. (amended) The method of claim 1 wherein said source of ATP is exogenous.

28
36. (amended) The method of claim ²⁶36 wherein said target 3' polyadenylated messenger RNA sequence is selected from the group consisting of an unlabeled target 3' polyadenylated messenger RNA sequence, a labeled target 3' polyadenylated messenger RNA sequence, and a [the]combination thereof.

29
37. (amended) The method of claim ²⁸36 wherein said labeled target 3' polyadenylated messenger RNA sequence is labeled with a moiety [is] selected from the group consisting of a fluorescent moiety, a visible moiety, a radioactive moiety, a ligand, and a combination of fluorescent and quenching moieties.

C13
38. (twice amended) The method of claim ²⁸36 wherein said determining the extent of deadenylation and degradation of said target 3' polyadenylated messenger RNA sequence comprises determining the extent of degradation of said labeled target 3' polyadenylated messenger RNA.

C14
39. (amended) The method of claim ²⁶36 wherein said RNA stability modifier increases the stability of said target 3' polyadenylated messenger RNA sequence.

32
40. (amended) The method of claim ³¹36 wherein said agent decreases the stability of said target 3' polyadenylated messenger RNA sequence increased by said RNA stability modifier.

33

26

41. (amended) The method of claim 33 wherein said RNA stability modifier decreases the stability of said target 3' polyadenylated messenger RNA sequence.

34

33

42. (amended) The method of claim 41 wherein said agent increases the stability of said target 3' polyadenylated messenger RNA sequence decreased by said RNA stability modifier.

36

35

44. (amended) The method of claim 43 wherein said AU rich element binding protein is selected from the group consisting of a member of the ELAV protein family; AUF1; tristetraprolin; AUH; TIA; TIAR; glyceraldehyde-3-phosphate; hnRNP C; hnRNP A1; AU-A; and AU-B.

42

46. (three times amended) A method for identifying an agent capable of modulating regulated deadenylation of a target 3' polyadenylated messenger RNA sequence comprising

(A) providing a system that recapitulates regulated RNA deadenylation of an exogenously added preselected target 3' polyadenylated messenger RNA sequence comprising

i) a cytoplasmic extract supernatant from a 100,000 x g, 1 hour centrifugation isolated from eukaryotic cells or tissues, said extract depleted of activity of proteins that bind polyadenylate;

ii) said target 3' polyadenylated messenger RNA sequence;

(B) introducing said agent into said system;

(C) monitoring the deadenylation of said target 3' polyadenylated messenger RNA sequence in said system; and

(D) identifying an agent able to modulate the extent of said deadenylation as capable of modulating the regulated deadenylation of said target 3' polyadenylated messenger RNA sequence.

38

47. (amended) A method for identifying an agent capable of modulating the deadenylation and degradation of a target 3' polyadenylated messenger RNA sequence comprising

(A) providing the system of claim 1;

- (B) introducing said agent into said system;
- (C) monitoring the deadenylation and degradation of said target 3' polyadenylated messenger RNA sequence in said system; and
- (D) identifying an agent able to modulate the extent of said deadenylation and degradation as capable of modulating the deadenylation and degradation of said target 3' polyadenylated messenger RNA sequence.

C17
~~48. (amended) A method for identifying an agent capable of modulating cell growth or cell differentiation in a mammal comprising determining the ability of said agent to modulate the stability of a target 3' polyadenylated messenger RNA sequence involved in the modulation of cell growth or differentiation in accordance with claim 19.~~

C18
~~51. (amended) A method determining whether an endogenous molecule participates in the deadenylation or degradation of RNA or regulation thereof comprising~~

- Subst
E13
~~(A) providing the system of claim 1 containing target 3' polyadenylated messenger RNA;~~
- ~~(B) introducing said endogenous molecule into said system; and~~
- ~~(C) monitoring the stability of said target 3' polyadenylated messenger RNA sequence in said system thereby determining whether said endogenous molecule is capable of modulating said regulation.~~

C19
~~53. (three times amended) A kit for monitoring the stability of a preselected exogenous target 3' polyadenylated messenger RNA sequence under conditions capable of recapitulating regulated RNA deadenylation and degradation, said kit comprising:~~

- ~~(a) a cytoplasmic extract supernatant from a 100,000 x g, 1 hour centrifugation, said extract depleted of activity of proteins that bind polyadenylate;~~
- ~~(b) other reagents; and~~
- ~~(c) directions for use of said kit.~~